REMARKS

Applicants thank Examiners Quang Nguyen and Dave Nguyen for the courtesy extended during a telephone conference on November 12, 2002 to discuss the outstanding claim rejections.

Applicants have canceled Claims 1-8, 10-18, 20, 21, 23-27, 29, 30, 32, 44, 46 and 51-54; amended Claims 33, 35, 37, 38, and 47; and added new Claims 55-58. Upon entry of this amendment, Claims 33-40, 42-43, 47-50, and 55-58 are pending. Each of the amendments is supported by the specification and claims as filed. Specifically, support for the recitation of "embryonic stem cells, embryonal carcinoma cells and embryonic gonadal cells or differentiated progeny thereof" can be found in the specification in the paragraph bridging pages 3 and 4. Support for the recitation of "mouse cells" can be found in the examples and at page 3, penultimate line. Support for the recitation of "viral replication factor" may be found in the specification at page 4, and support for episomal maintenance of the vector may be found on pages 3 and 12. Support for the recitation of "transgenic mouse cell" in Claim 47 may be found in the specification at page 7. The first full paragraph on page 7 explains how the replication factor can be supplied on a construct that integrates into a genome, indicating clearly that a transgenic mouse is contemplated. The viral replication factors of Claim 55 are supported in the specification at page 4. Claim 56 is supported in the specification at page 14. Claim 57, reciting the step of isolating DNA, is supported by discussion of

identification of DNA's in the specification, for example, by reference to screening of cDNA libraries at the bottom of page 11. Basis for Claim 58 is found in original Claim 34. None of the amendments adds new matter.

PRIORITY

Applicants will provide a certified copy of the priority application within the next ten days. Applicants have also amended the specification to recite the correct filing date for PCT/GB98/00216.

CLAIM OBJECTIONS

Claims 8 and 44, objected to by the Examiner have been canceled.

WRITTEN DESCRIPTION

The Examiner has rejected the claims under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time the application was filed. Applicants request reconsideration and withdrawal of this rejection.

The pending claims are directed to assays in mouse cells selected from embryonic stem cells, embryonal carcinoma cells, embryonic gonadal cells, or differentiated derivatives thereof. The Examiner acknowledges at page 4 of the Office Action that this subject matter is disclosed in the specification. Accordingly, the rejection should be withdrawn.

ENABLEMENT

The claims stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly not enabled by the specification. The Examiner acknowledges at page 8 of the Office Action that the specification is enabling for in vitro methods of obtaining a gene product by expressing a DNA in mouse ES, EC, or EG cells transfected with a viral replication factor. The pending claims are directed to this subject matter and therefore, the rejection should be withdrawn.

CLARITY

Claims 1-8, 10-16, 18, 33-40, 42-44, and 46-50 are rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite. Claims 1-8, 10-16, 18, 44, and 46 have been canceled, thereby obviating the rejection as to these claims.

The Examiner contends that the phrase "otherwise obtaining a cell that expresses or will express the replication factor" in step (a) of Claims 33 and 47 renders those claims unclear. Amended claims 33 and 47 avoid the use of this phrase, thereby overcoming the Examiner's concern.

Claim 35 was rejected as allegedly unclear due to the phrase "the effect of simultaneous presence in the cell of a first test factor and a second test factor." Claim 35 now recites a further positive step of transfecting the mouse with a third vector containing a second test factor, thereby rendering the claim sufficiently clear.

The Examiner contends that the meaning of "linked to" in Claim 37 is unclear. Applicants have obviated the Examiner's concern by amending Claim 37 to recite "operatively linked to."

Claim 38 is rejected as allegedly omitting an essential step. Applicants have amended Claim 38 to delete the term "optionally." As amended, Claim 38 includes the positive step of determining whether the cell active protein is transported to the cell surface and remains there or is secreted by the cell.

In view of the foregoing, Applicants request that the rejections under 35 U.S.C. §112, second paragraph, be withdrawn.

ANTICIPATION

Claims 1-5, 8, 10-12, and 54 have been canceled, thereby obviating these rejections under 35 U.S.C. § 102.

OBVIOUSNESS

Claims 1, 6-7, 14-15, 17-25, 27, 29-30, 32, 44, 46, and 51-54 have been canceled, rendering the rejection of these claims moot.

Claims 33 to 36 and 50 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over *Carstens et al.* (Gene 164:195-202, 1995) in view of *Gassman et al.* (Proc. Natl. Acad. Sci. 92:1292-1296, 1995) and *Sambrook et al.* (Molecular Cloning: A Laboratory Manual, Sections 16.8-16.9, 1989). Claims 37 to 40, 45, and 47-50 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over *Tashiro et al.* (Science 261:600-603, 1993) in view of *Carstens et al.* (Gene 164:195-202, 1995), *Sambrook et al.* (Molecular Cloning: A Laboratory Manual, Sections 16.8-16.9, 1989), *Gassman et al.* (Proc. Natl. Acad. Sci. 92:1292-1296, 1995), *Williams et al.* (Nature 336:684-687, 1988), and *Moreau et al.* (Nature 336:;690-692, 1988). Applicants traverse.

The pending claims specify that the cells used in the methods of the invention are mouse cells. *Carstens et al.* explicitly teach that their shuttle vector-based systems do not operate on rodent cells, rodent including mouse and rat cells. See, specifically, *Carstens et al.*, page 196, right hand column, results and discussions section where it is stated that "EBV-based shuttle vector systems will replicate in most mammalian cells (with the exception of rodent cells). . . ."

Carsten et al. also state (at page 198, right hand column, middle of the page):

> However, after selection for the library vector and subsequent selection for ANC-I growth, we encountered problems with senescence, severely limiting the usefulness as a host strain. Since rodent cell lines do not support replication of EBV-based shuttle vectors, this required the use of an immortalised human fibroblast cell line which does not display transformed growth characteristics.

It is thus apparent that *Carstens et al.* does not provide a teaching that is relevant to expression in mouse cells because the shuttle vector system of *Carstens et al.* specifically does not work in mouse cells. *Carstens et al.* were forced to use a particular, immortalised human fibroblast cell line.

In contrast, *Gassmann et al.* relates to plasmid vectors in mouse embryonic stem cells. It is apparent, from even a cursory review, that these documents cannot be read together. One of skill in the art would not be motivated to combine the teachings of *Gassman et al.* and *Carstens et al.* in the manner suggested by the Examiner.

The citation of *Sambrook et al.* does nothing to remedy the deficiencies already noted and, in fact, further distinguishes the cited references from the claimed invention. Specifically, Claim 33 recites that both the first vector and the second vector are maintained episomally in the mouse cells in the assay of the invention. Claim 33 also

indicates that the viral replication factor of the first vector maintains the second vector episomally. Section 16.8 in *Sambrook et al.*, cited by the Examiner states:

> Plasmids bearing these viral replicons are replicated episomally as long as the appropriate *trans*-acting factors are provided by genes either carried on the plasmid or within the genome of the host cell.

In contrast, in the method of Claim 33, the replication factor is not provided on the genome of the host cell but rather, it is provided episomally. The alternative offered by *Sambrook et al.* is for the factor to be provided on the plasmid, that is to say on the plasmid that is to be replicated. In contrast, in the method of Claim 33, the factor is not provided on the plasmid that is to be replicated. Instead, it is provided on a separate vector.

Similarly, the citation of *Tashiro et al.*, *Williams et al.*, and *Moreau et al.* does nothing to remedy the defects in the first combination of references cited by the Examiner. Moreover, the method of Claim 37 explicitly recites the step of "determining if the cell differentiates". The Examiner has acknowledged that *Tashiro et al.*, *Williams et al.*, and *Moreau et al.* does not teach a system wherein the expressed cell active protein inhibits differentiation of the transfected cell. This deficiency is not remedied in the other art cited by the Examiner, as none of the other art teaches that a useful marker for whether a cell active protein has been transported to a cell surface is observation of whether or not a cell differentiates. Although factors that inhibit or promote differentiation may be known, there is no teaching that observation of whether or not a cell differentiates can be used as a key step in an assay for a polypeptide that directs transport of a cell active protein to a cell surface.

The combination of documentd cited by the Examiner does render the claimed invention prima facie obvious. Accordingly, the rejections of the claims under 35 U.S.C. §103(a) should be withdrawn.

Camenisch et al.

Applicants have previously drawn the attention of the Examiner to a paper by *Camenisch et al.* (See, Information Disclosure Statement filed March 18, 2002.) This paper names Gassmann as a co-author. *Camenisch et al.* represents further development of the research reported in *Gassmann et al.* Applicants believe that *Gassmann et al.* can not be read in isolation. The later paper by *Camenisch et al.* provides an insight into the direction taken by *Gassmann et al.* following their initial work on maintenance of extrachromosomal plasmid vectors in mouse embryonic stem cells. The *Camenisch et al.* paper demonstrates that the direction taken by *Camenisch et al.*, the skilled men in the art, was to use a single vector system for expression of genes of interest. Following the one vector approach of *Camenisch et al.*, a single transfection event is required in order to express a gene of interest.

According to the present invention, two transfection events are required to express a gene of interest. The first transfection uses a first vector which expresses a viral replication factor. This first vector does not express the gene of interest. The second transfection uses a second vector which expresses the gene of interest but which is dependent upon expression of the viral replication factor by the first vector in order for the second vector to be maintained episomally in the mouse cell.

The Examiner has not indicated why it would be obvious to adopt a two vector system for expression of a gene of interest when *Camenisch et al.* teaches that this can be achieved using a single vector system. This invention provides, for the first time, an efficient method for assay of biological effect of genes of interest. This invention teaches that a first transfection step can be used to generate a pool of transfected mouse cells which express the viral replication factor from an episomally maintained vector. This pool of cells can then be divided and used for subsequent transfections with different second vectors each containing a different gene of interest. This combination of first and second transfection steps enables an assay of biological effect of genes of interest to be carried out efficiently. The process is efficient because the second transfection step is relatively easier to perform than the first transfection step due to the pre-existence within the transfected cell of the first vector which expresses the viral replication factor. This efficiency would not have been obvious from the *Gassmann et al.* in view of the continued work reported in *Camenisch et al.* because *Camenisch et al.* teaches use of a one vector system only.

In this light, the Examiner is asked to acknowledge the non-obviousness of all pending claims.

CONCLUSION

In view of the foregoing amendments and remarks, Applicants respectfully request the reconsideration and reexamination of this application and the timely allowance of the pending claims.

Please grant any extensions of time required to enter this Amendment that are not already accounted for by the accompanying Petition for Extension of Time and charge any additional required fees to deposit account 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

Dated: December 4, 2002

By: _____
Leslie A. McDonell
Reg. No. 34,872

CERTIFICATE OF TRANSMISSION UNDER 37 CFR § 1.8
I hereby certify that this correspondence is being transmitted by facsimile to the Patent and Trademark Office on December 4, 2002.
By: Rachel Venturi

APPENDIX TO AMENDMENT
Version with Markings to Show Changes Made

CHANGES TO THE SPECIFICATION:

--This is a continuation of application Serial No. PCT/GB98/00216, filed January 26 [24], 1997, [-- all of] which is [are] incorporated herein by reference. --

CHANGES TO THE CLAIMS:

33. (Twice Amended) An in *vitro* assay for a biological [the] effect of presence of a protein or polypeptide or other product of DNA expression in a mouse embryonic stem (ES) cell, a mouse embryonal carcinoma (EC) cell or a mouse embryonic gonadal (EG) cell, or a differentiated derivative thereof [cell, selected from the group consisting of an ES cell, an EC cell and an EG cell, of a protein or polypeptide or other product of DNA expression], comprising the steps:

(a) [(i)] transfecting the mouse cell with a first episomal vector that expresses a viral replication factor; [or

(ii) otherwise obtaining a cell that expresses or will express the replication factor;]

(b) transfecting the mouse cell of step (a) with a second vector, wherein

(i) the second vector contains a DNA coding for the protein or polypeptide or other product of DNA expression in operative combination with a promoter for expression of the DNA;

(ii) the second vector also contains a DNA coding for a selectable marker in operative combination with a promoter for expression of the selectable marker; and

(iii) the viral replication factor of step (a) replicates the second vector episomally [extrachromosomal replication of the second vector is dependent upon presence within the cell of the replication factor];

(c) isolating mouse cells of step (b) [selecting for cells that have been transfected with the second vector]; and

(d) maintaining the isolated mouse [selected] cells over a plurality of generations so as to assay the biological effect of expression of the protein or polypeptide or other product of DNA expression.

34. The assay according to Claim 33, wherein step (a) is carried out once and the cells obtained are divided and used for a plurality of separate assays in which steps (b)-(d) are carried out a plurality of times with second vectors containing different DNA sequences.

35. (Three Times Amended) The assay according to Claim 33, for assay of a biological [the] effect of simultaneous presence of a first protein, polypeptide or other product of DNA expression and a second protein, polypeptide or other product of DNA expression in a mouse ES cell, a mouse EC cell or a mouse EG cell, or a differentiated

derivative thereof, further comprising the step of transfecting the mouse cell of step (b) with a third vector, wherein the third vector contains

(1) a DNA coding for the second protein, polypeptide or other product of DNA expression in operative combination with a promoter for expression of the DNA, and

(2) a DNA coding for a selectable marker in operative combination with a promoter for expression of the selectable marker,

wherein the viral replication factor of step (a) replicates the third vector episomally [in the cell of a first test factor and a second test factor, wherein said first test factor and second test factors are independently selected from the group consisting of a protein, a polypeptide and another product of DNA expression].

36. A method of screening a library of cDNAs comprising assaying the effect of expression of each of the cDNAs according to the method of Claim 33.

37. (Three Times Amended) An *in vitro* method of assaying whether a DNA under investigation codes for a polypeptide that directs transport of a cell active protein to a cell surface comprising the steps of:

(a) expressing a composite DNA including (a) a DNA sequence under investigation, operatively linked to (b) a DNA coding for the cell active protein in a mouse cell, selected from the group consisting of an ES cell, an EC cell, an EG cell, and differentiated progeny thereof [a composite DNA including (a) a DNA sequence under investigation, linked to (b) a DNA coding for the cell active protein], wherein

(i) activity of the cell active protein is dependent upon transport of the cell active protein to the cell surface,

(ii) the DNA of (b) does not code for a polypeptide that directs transport of the cell active protein to the cell surface, and

(iii) the cell active protein inhibits differentiation of the cell and in the absence of the cell active protein the cell will differentiate; and

(b) determining if the cell differentiates.

38. (Twice Amended) The method according to Claim 37 wherein the DNA under investigation is [for screening in] a library of DNAs, which is screened to identify DNA sequences coding for signal polypeptide sequences that transport proteins to the cell surface, and wherein the method [optionally] comprises the additional step of determining whether the cell active protein is transported to the cell surface and remains there or is secreted by the cell.

39. The method according to Claim 37 wherein the DNA of (b) is obtained by deleting or disabling, from a DNA encoding a cell surface or secreted protein, that portion of the DNA that codes for the polypeptide sequence responsible for transportation of the protein to the cell surface.

40. The method according to Claim 37 wherein the cell active protein induces a morphological or proliferative change in the cell.

42. The method according to Claim 37 wherein the cell active protein is a cell surface receptor.

43. The method according to Claim 42 wherein the cell active protein is an IL-6 receptor and the DNA of (b) encodes a modified form of the receptor preprotein lacking a functional signal sequence.

47. (Three Times Amended) The method according to Claim 37, wherein [comprising expressing] the composite DNA is expressed by:

(a) (i) transfecting the mouse cell with a first vector that expresses a viral replication factor; or

(ii) otherwise obtaining a transgenic mouse cell, selected from the group consisting of an ES cell, an EC cell and an EG cell, that expresses [or will express] the replication factor;

(b) transfecting the mouse cell of step (a) with a second vector, wherein

(i) the second vector contains the composite DNA in operative combination with a promoter for expression of the composite DNA;

(ii) the second vector also contains a DNA coding for a selectable marker in operative combination with a promoter for expression of the selectable marker; and

(iii) the viral replication factor of step (a) replicates the second vector episomally [extrachromosomal replication of the second vector is dependent upon presence within the cell of the replication factor];

(c) isolating mouse cells from step (b) [selecting for cells that have been transfected with the second vector]; and

(d) maintaining the isolated mouse [selected] cells over a plurality of generations so as to assay the effect of expression of the composite DNA.

48. The method according to Claim 47 wherein step (a) is carried out once and the cells obtained are divided and used for a plurality of separate methods in which steps (b)-(d) are carried out a plurality of times with second vectors containing different DNA sequences.

49. The method according to Claim 37 for identification of a DNA coding for a cell surface or secreted protein comprising isolating the DNA under investigation.

50. The method according to Claim 37 for identification of a cell surface or secreted protein comprising isolating a protein or polypeptide encoded by the DNA under investigation.

55. (New) The assay according to Claim 33, wherein the viral replication factor is a viral replication factor selected from polyoma large T antigen, EBNA-1 antigen, papilloma virus replication factors, and SV40 large T antigen.

56. (New) The assay according to Claim 33, wherein said second vector contains a DNA that codes for an anti-sense RNA.

57. (New) The assay according to Claim 33, further comprising the step: (e) isolating said DNA coding for the protein or polypeptide or other product of DNA expression.

58. (New) An in *vitro* assay for a biological effect of presence of first and second products of DNA expression in mouse embryonic stem (ES) cells, mouse embryonal carcinoma (EC) cells or mouse embryonic gonadal (EG) cells, comprising the steps:

(a) transfecting the mouse cells with a first vector that expresses a viral replication factor;

(b) isolating cells of step (a) and dividing the cells into at least a first sub-population of transfected cells and a second sub-population of transfected cells;

(c) transfecting the first sub-population of transfected cells with a second vector, wherein the second vector contains a DNA coding for a first product of DNA expression in operative combination with a promoter for expression of the DNA and also contains a DNA coding for a selectable marker in operative combination with a promoter for expression of the selectable marker, and wherein the viral replication factor of step (a) replicates the second vector episomally;

(d) isolating cells of step (c);

(e) maintaining the isolated cells of step (d) over a plurality of generations so as to assay the biological effect of expression of the first product of DNA expression;

(f) transfecting the second sub-population of transfected cells with a third vector, wherein the third vector contains a DNA coding for a second product of DNA expression in operative combination with a promoter for expression of the DNA and also contains a DNA coding for a selectable marker in operative combination with a promoter for expression of the selectable marker, and wherein the viral replication factor of step (a) replicates the third vector episomally;

(g) isolating cells of step (f); and

(h) maintaining the isolated cells of step (g) over a plurality of generations so as to assay the biological effect of expression of the second product of DNA expression.